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CLINICAL CASE SEMINAR

$G_s\alpha$ Mutation at Codon 201 in Pituitary Adenoma Causing Gigantism in a 6-Year-Old Boy with McCune-Albright Syndrome*

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In children, pituitary tumors causing gigantism are very rare, and $G_s\alpha$ mutations in tumor tissue have not been reported.

A 6.5-yr-old boy was referred with a body height of 149.7 cm (SD score, +5.9) and a growth velocity of 13 cm/yr (>99th percentile). Insulin-like growth factor I (IGF-I) and IGF-binding protein-3 (IGFBP-3) levels were 753 μg/L and 5.4 mg/L, respectively (>99th percentile), and GH levels were not suppressible by glucose (minimal level, 37 μg/L). The basal PRL level was 1316 mU/L. A cranial magnetic resonance imaging (MRI) scan revealed a 1.8 × 1.8-cm pituitary tumor. Osseous lesions suggestive of fibrous dysplasia were seen on MRI scan (occipital scull) and x-rays (humerus). After pretreatment with octreotide, transnasal surgery was performed. A pituitary adenoma was largely removed, as evidenced by MRI imaging and a decrease in/normalization of GH, PRL, IGF-I, and IGFBP-3 levels.

Tumor tissue stained positive for GH and PRL immunoreactivity. In tumor tissue DNA a single point mutation leading to an exchange of Arg to Cys at codon 201 of $G_s\alpha$ was revealed by PCR and automated sequencing. Restriction analysis discriminating between the mutated and nonmutated allele confirmed the mutation. We hypothesize that the mutation led to the development of McCune-Albright syndrome (MAS) with pituitary adenoma, causing gigantism in this child.

Mutations of the α-subunit of stimulatory GTP-binding proteins, $G_s\alpha$, have been shown to be related to the development of pituitary adenomas (1, 2). The mutation leads to constitutive activation of the G_s proteins, causing increased adenylate cyclase activity. This increased second messenger activation is thought to be tumorigenic (3, 4). Activating point mutations for the human $G_s\alpha$ gene have been shown in codon 201 (Arg) and 227 (Glu) of exons 8 and 9, respectively. The oncogene generated by these mutations is termed *gsp*. In children, pituitary tumors causing gigantism are very rare, and $G_s\alpha$ mutations in tumor tissue have not been reported.

Another rare condition associated with mutations of the $G_s\alpha$ gene is the MAS (5, 6). MAS is a sporadic disease characterized by polyostotic fibrous dysplasia, café-au-lait spots, precocious puberty, and multiple endocrinopathies. Affected tissues in patients with MAS have been shown to be *gsp* positive (5–7).

Case report

A 6.5-yr-old boy was presented with a body height of 149.7 cm (SD score, +5.9; Fig. 1) (8) and a growth velocity of 13 cm/yr (>99th percentile). Signs of precocious puberty were found (pubes II, testicular volume, 4 mL) (9). No café-au-lait spots and no symptoms or signs of other pituitary hormone disturbances were found. Target height was 177 cm (44th percentile). The mother's menarche had occurred at 13 yr.

When first seen, the patient's bone age was accelerated by 3.5 yr (Fig. 1) (10). IGF-I and IGFBP-3 were far above the 99th percentile, and basal GH and PRL levels were elevated (Table 1A). GH was not suppressible by oral glucose tolerance test. Other endocrine function tests were normal or only slightly beyond the normal range (Table 1A). A MRI scan of the brain showed a 1.8 × 1.8-cm pituitary tumor (Fig. 2A) and a squamous osseous occipital lesion. The latter as well as an osseous lesion of the right humerus seen on x-ray were suggestive of fibrous dysplasia. Perimetry was normal.

Therapy with octreotide (two daily doses of 100 μg, sc) was started, decreasing the growth rate to 3 cm/yr and slightly lowering IGF-I, IGFBP-3, and GH levels (Table 1B). Enucleation of the adenoma (Fig. 2B) lead to near normalization of PRL and GH, and a slow but steady decline of IGF concentrations (Table 1B). Three months after surgery, the growth rate rose again to 12 cm/yr. Currently, under treatment with two daily doses of 100 μg octreotide, sc, growth rate has dropped to 4.2 cm/yr (GH and IGFs; Table 1B).

Materials and Methods

Tissue origin and immunostaining

Tumor tissue obtained at surgery was immediately frozen in liquid nitrogen for hormone immunostaining and DNA extraction. Frozen sections of tumor tissue were prepared and stained for GH, PRL, TSH, FSH, LH, and ACTH immunohistochemistry.

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DNA preparation, PCR techniques, and sequencing

DNA was prepared from homogenized tumor tissue under standard conditions, using cell lysis and DNA preparation techniques (11). Lymphocyte DNA of the patient was extracted with a commercial kit (QIAamp blood kit, Quiagen, Hilden, Germany). PCR was carried out with 0.2 μg genomic DNA using *Taq* polymerase (Boehringer, Mannheim, Mannheim, Germany) and 0.8 μmol/L of the respective oligonucleotide primers in a volume of 100 μL and a deoxynucleotide concentration of 0.5 mmol/L. The following primer pairs for $G_s\alpha$ were used (12): exon 8 (210 bp), 5′-CTACTCCAGACCTTTGCTTTAG-3′ (P1) and 5′-ACAGCTGGTTATTCCAGAGGGA-3′ (P2); and exon 9 (170 bp), 5′-GTTTCTTGACATTCACCCCAGT-3′ (P3) and 5′-AGCGACCCTGATCCCTAACAAC-3′ (P4). Forty PCR cycles were carried out in a Trioblock (Biometra, Göttinger, Germany) as follows: 90 s at 94 C, 90 s of annealing at 60 C, and 60 s of extension at 72 C.

The PCR products corresponding to the predicted lengths of 210 bases (exon 8) and 170 bases (exon 9) were ligated into a T-tailed pGEM5 plasmid (Promega, Madison, WI). After transfection in *Escherichia coli* (JM 109, Promega), eight recombinant colonies from the patient's lymphocyte and pituitary tumor DNA, respectively, were sequenced using an automated sequencer (Perkin-Elmer, Weiterstadt, Germany).

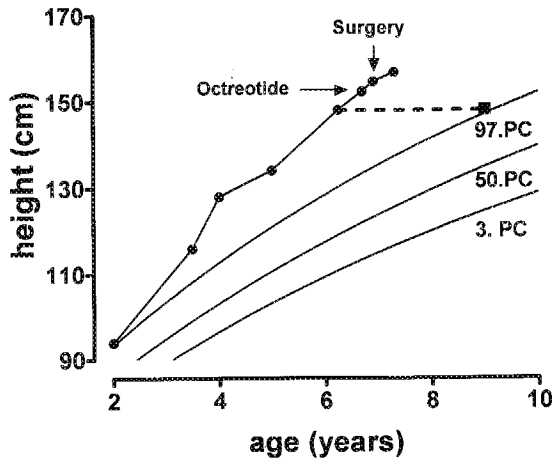

FIG. 1. Growth curve of a 6.5-yr-old boy with gigantism due to a mixed GH- and PRL-secreting pituitary adenoma with a $G_s\alpha$ mutation at codon 201 in exon 8. Third, 50th, and 97th percentiles are shown. The patient's length is represented by *circles*; bone age is shown by the *square*.

Restriction enzyme digestion

For detection of the C to T mutation in base 442 (DNA sequence from the EMBL Gene Bank, Heidelberg, Germany; access no. M21142) (13), a 258-bp segment of $G_s\alpha$ was amplified. In one of the primers, a thymidine, normally present at position 439 in the mutated and wild-type genes, was replaced by adenine. This change generated a specific recognition site for the *Pvu*II restriction enzyme (Life Technologies, Eggenstein, Germany), yielding two fragments (235 and 23 bp) when base 442 of $G_s\alpha$ was mutated from C to T. The primer sequences were 5′-TTGTTTCAGGACCTGCTTCGCAGC-3′ (P5) and 5′-GGTTATTCCAGAGGGACTGGGGTGA-3′ (P6). Similarly, a specific recognition site for the *Xma*III restriction enzyme was created, yielding 22- and 236-bp fragments when no mutation in base 442 was present. The primer sequences were 5′-TTTGTTTCAGGACCTGCTTCGCGGC-3′ (P7) and 5′-GGTTATTCCAGAGGGACTGGGGTGA-3′ (P8).

After PCR amplification and incubation with the appropriate restriction enzyme, the digested samples were analyzed by gel electrophoresis on a 3% agarose gel (MetaPhor, Biozym, Oldendorf, Germany).

Results

Immunohistochemical stains were positive for GH and PRL (20% and 10% of the cells, respectively).

PCR analysis of genomic $G_s\alpha$ DNA from the patient's tumor tissue revealed a single point mutation of C to T at position 442 in exon 8, leading to a base exchange of arginine to cysteine in codon 201 of $G_s\alpha$ in five of eight PCR-generated clones. No mutation was found in exons 8 and 9 of DNA from the patient's lymphocytes.

The sequencing results were confirmed by restriction enzyme digestion (Fig. 3).

Discussion

A mixed GH- and PRL-secreting pituitary adenoma with a $G_s\alpha$ mutation of one allele at codon 201 caused gigantism in a 6.5-yr-old boy. GH-secreting tumors are very rare in childhood, and less than 15 cases have been reported to date

TABLE 1A. Endocrine function tests in a 6.5-yr-old patient with gigantism at first presentation

	First presentation (3/95)
IGF-I (μg/L); / 5th–95th percentile, 54–220)	753 (>99th percentile)
IGF-BP3 (mg/L; /5th–95th percentile, 1.7–3.6)	5.4 (>99th percentile)
Basal GH (μg/L; normal, <5 μg/L)	44.9
PRL (mU/L; normal, 20–350 mU/L)	1316
fT_3, fT_4, TSH (normal, 2–4.5 ng/L, 8–20 ng/L, 0.5–4 mU/L, respectively)	3 ng/L, 13 ng/L, 1.6 mU/L
Testosterone (ng/L; normal, 40–110 ng/L)	380
17-Hydroxyprogesterone (ng/L; normal, <630 ng/L)	1200
Oral glucose tolerance test (minimal and basal GH, μg/L; normal, <2 μg/L)	37 and 44.9
CRH test (cortisol before and after CRH; normal, >20 μg/L)	39 and 85 μg/L
TRH test (TSH before and after TRH; normal after TRH, >2.5 mU/L	TSH: 1.1 and 7.9 mU/L
LHRH test (before and after LHRH)	LH: 0.1 and 0.5 mU/L FSH: <0.2 and 1.1 mEq/mL
GH-binding protein (pmol/L; normal, 200–400 pmol/L)	299

TABLE 1B. Endocrine function in a 6.5-yr-old patient with gigantism during the course of treatment

	After octreotide treatment (2 × 100 μg; 6/95)	After partial adenomectomy (9/95)	During second octreotide treatment (2 × 100 μg; 1/96)
IGF-I (μg/L)	532 (>99th percentile)	502 (>99th percentile)	371 (>99th percentile)
IGFBP-3 (mg/L)	4.8 (>99th percentile)	5.0 (>99th percentile)	3.6 (95th percentile)
Basal GH (μg/L)	3.6	11.4	1.8
PRL (mU/L)	864	150	148
GHBP (pmol/L)	578	327	

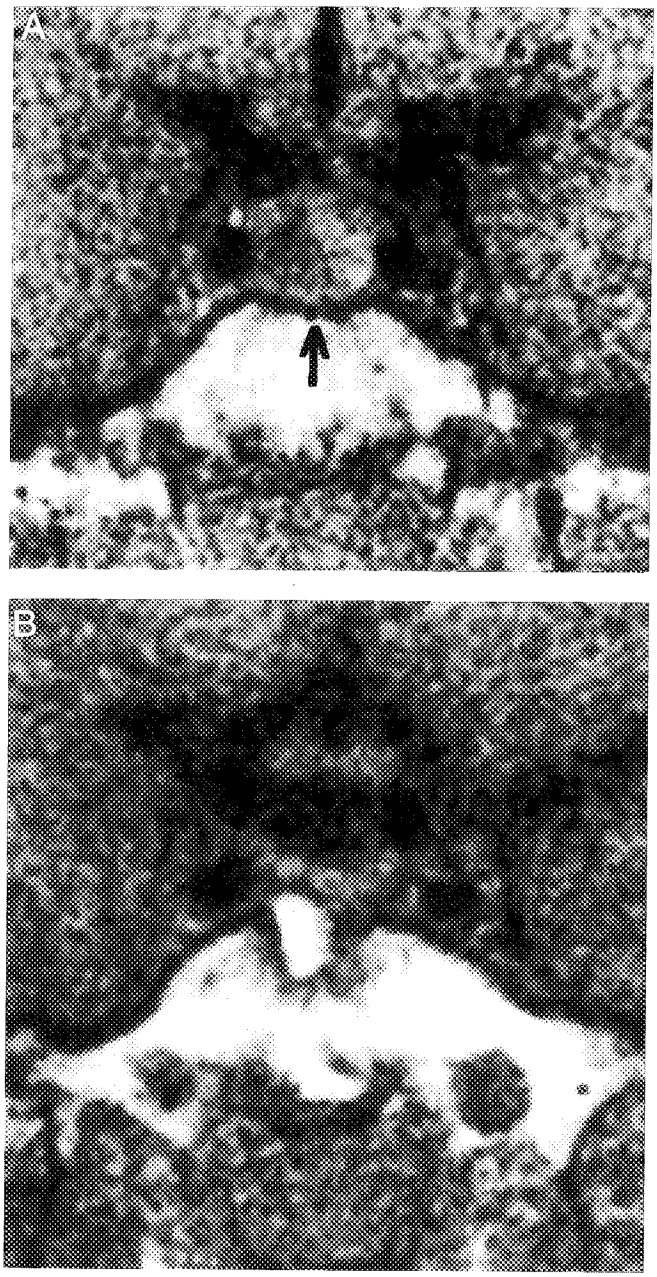

FIG. 2. Cranial MRI of a 6.5-yr-old patient with gigantism showing a 1.8 × 1.8-cm cystic adenoma of the pituitary gland. A, preoperative MRI; B, MRI 2 months after enucleation of the adenoma.

(12, 14). In none of these children was a $G_s\alpha$ mutation detected, whereas in adult acromegalic patients from Western countries, the frequency of a mutation of $G_s\alpha$ is 30–45% in GH-secreting adenomas (1, 2, 15). A recent meta-analysis on 138 adults with a GH-producing adenoma suggests that *gsp*-positive patients are significantly older than *gsp*-negative adults (16), which may partially account for the fact that this mutation has not been found in tumor tissue of children until now.

However, the finding of a *gsp*-positive adenoma in our patient also has to be seen in context with the presence of MAS in this patient. MAS is known to be associated with

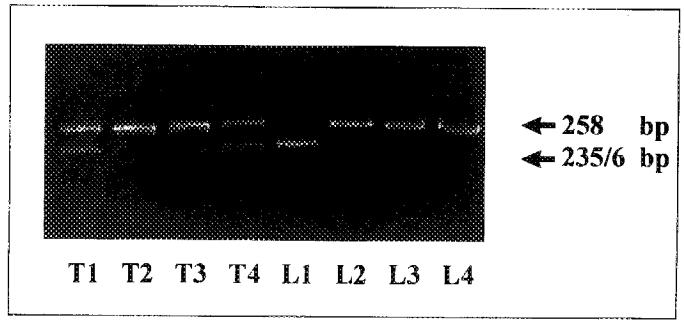

FIG. 3. Restriction enzyme digestion of a 258-bp fragment amplified from exon 8 of $G_s\alpha$. Primers P5 and P6 inserted a mutation, making the amplified fragment susceptible for digestion by *Pvu*II if a C to T mutation leading to a base exchange of arginine to cysteine in codon 201 was present, yielding a DNA fragment with a length of 235 bp. Primers P7 and P8 introduced a restriction site for *Xma*III if no mutation was present, yielding a DNA fragment with a length of 236 bp. The results indicate a mutation in base 442 in 50% of the tumor tissue DNA and a complete absence of the mutation in base 442 in DNA from the patient's lymphocytes. L, DNA obtained from lymphocytes; T, DNA obtained from tumor tissue. 1: P7, P8, *Xma*III; 2: P7, P8, *Pvu*II; 3: P5, P6, *Xma*III; 4: P5, P6, *Pvu*II.

endocrine abnormalities, including pituitary tumors. In the pituitary adenomas of two patients with MAS, aged 17 and 33 yr, a $G_s\alpha$ mutation was found (6, 7). In one of these patients, the mutation was also found in osseous lesions (6). Other affected tissues of patients with MAS are usually *gsp* positive as well, but no mutations are found in the patients' blood (6). This observation is consistent with the absence of a $G_s\alpha$ mutation in the lymphocytes from our patient. In the present study, biopsies from tissues other than the pituitary gland were not justified for diagnostic or therapeutic reasons.

In summary, we hypothesize that the single point mutation in exon 8 of $G_s\alpha$ led to the development of MAS with pituitary adenoma causing gigantism in this child.

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